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Abstract: The interaction between metal ions and nucleotides is well characterized, as is their importance for metabolic processes, e.g. in the synthesis of nucleic acids. Hence, it is surprising to find that no detailed comparison is available of the metal ion-binding properties between nucleoside 5'-phosphates and 2'-deoxynucleoside 5'-phosphates. Therefore, we have measured here by potentiometric pH titrations the stabilities of several metal ion complexes formed with 2'-deoxyadenosine 5'-monophosphate (dAMP²⁻), 2'-deoxyadenosine 5'-diphosphate (dADP³⁻) and 2'-deoxyadenosine 5'-triphosphate (dATP⁴⁻). These results are compared with previous data measured under the same conditions and available in the literature for the adenosine 5'-phosphates, AMP(2-), ADP(3-) and ATP(4-), as well as guanosine 5'-monophosphate (GMP(2-)) and 2'-deoxyguanosine 5'-monophosphate (dGMP(2-)). Hence, in total four nucleotide pairs, GMP(2-)/dGMP(2-), AMP(2-)/dAMP(2-), ADP(3-)/dADP(3-) and ATP(4-)/dATP(4-) (= NP/dNP), could be compared for the four metal ions Mg²⁺, Ni²⁺, Cu²⁺ and Zn²⁺ (= M²⁺). The comparisons show that complex stability and extent of macrochelate formation between the phosphate-coordinated metal ion and N7 of the purine residue is very similar (or even identical) for the AMP(2-)/dAMP(2-) and ADP(3-)/dADP(3-) pairs. In the case of the complexes formed with ATP(4-)/dATP(4-) the 2'-deoxy complexes are somewhat more stable and show also a slightly enhanced tendency for macrochelate formation. This is different for guanine nucleotides: the stabilities of the M(dGMP) complexes are clearly higher, as are the formation degrees of their macrochelates, than is the case with the M(GMP) complexes. This enhanced complex stability and greater tendency to form macrochelates can be attributed to the enhanced basicity (ΔpK_a 0.2) of N7 in the 2'-deoxy compound. These results allow general conclusions regarding nucleic acids to be made.

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Effect of the ribose versus 2'-deoxyribose residue on the metal ion-binding properties of purine nucleotides

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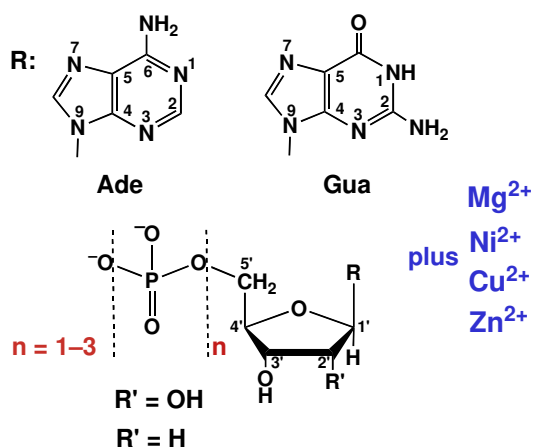
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Graphical contents entry



Among the 2'-deoxyribose nucleotides (dNMP²⁻, dNDP³⁻, dNTP⁴⁻) the complexes of dGMP²⁻ and to a smaller extent also of dATP⁴⁻ are somewhat more stable than the corresponding ribose nucleotides (NMP²⁻, NDP³⁻, NTP⁴⁻) and macrochelate formation involving N7 is also more pronounced.

Summary

The interaction between metal ions and nucleotides is well characterized, as is their importance for metabolic processes, e.g., in the synthesis of nucleic acids. Hence, it is surprising to find that no detailed comparison is available of the metal ion-binding properties between nucleoside 5'-phosphates and 2'-deoxynucleoside 5'-phosphates. Therefore, we have measured here by potentiometric pH titrations the stabilities of several metal ion complexes formed with 2'-deoxyadenosine 5'-monophosphate (dAMP²⁻), 2'-deoxyadenosine 5'-diphosphate (dADP³⁻) and 2'-deoxyadenosine 5'-triphosphate (dATP⁴⁻). These results are compared with previous data measured under the same conditions and available in the literature for the adenosine 5'-phosphates, AMP²⁻, ADP³⁻ and ATP⁴⁻ as well as guanosine 5'-monophosphate (GMP²⁻) and 2'-deoxyguanosine 5'-monophosphate (dGMP²⁻). Hence, in total four nucleotide pairs, GMP²⁻/dGMP²⁻, AMP²⁻/dAMP²⁻, ADP³⁻/dADP³⁻ and ATP⁴⁻/dATP⁴⁻ (= NP/dNP), could be compared for the four metal ions Mg²⁺, Ni²⁺, Cu²⁺ and Zn²⁺ (= M²⁺). The comparisons show that complex stability and extent of macrochelate formation between the phosphate-coordinated metal ion and N7 of the purine residue is very similar (or even identical) for the AMP²⁻/dAMP²⁻ and ADP³⁻/dADP³⁻ pairs. In the case of the complexes formed with ATP⁴⁻/dATP⁴⁻ the 2'-deoxy complexes are somewhat more stable and show also a slightly enhanced tendency for macrochelate formation. This is different for guanine nucleotides: At least the stabilities of the M(dGMP) complexes are clearly higher, as are the formation degrees of their macrochelates, than it is the case with the M(GMP) complexes. This enhanced complex stability and enlarged tendency to form macrochelates can be attributed to the enhanced basicity (ΔpK_a ca. 0.2) of N7 in the 2'-deoxy compound. These results allow general conclusions regarding nucleic acids.

1 Introduction

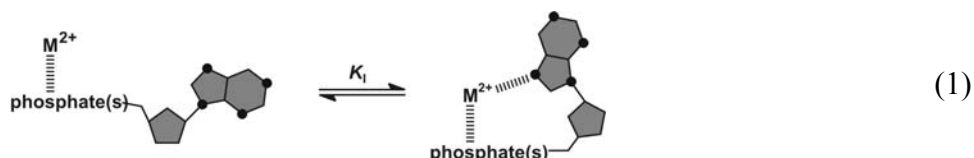
Nucleotides participate in all kinds of metabolic processes,¹ adenosine 5'-triphosphate (ATP^{4-}) and guanosine 5'-triphosphate being especially prominent.² Nucleoside 5'-triphosphates (NTPs) as well as most other nucleotides serve as substrates only in the form of their metal ion complexes (see refs. 2,3) including the formation of nucleic acids as catalyzed by polymerases.⁴ The sugar moieties that occur overwhelmingly in nature are ribose and 2'-deoxyribose residues.⁵ Consequently, the nucleotide building blocks give rise to two types of nucleic acids, the ribonucleic acids (RNA) and the 2'-deoxyribonucleic acids (DNA).⁵ The latter ones, which miss the 2'-hydroxy group, are less sensitive to hydrolysis at the phosphate-diester backbone than are RNAs.⁶

For nucleotides it has very recently been shown⁷ that the presence or absence of the 2'-OH group affects the acid-base properties somewhat: 2'-deoxynucleoside 5'-phosphates ($\text{dNP}^{2-/3-/4-}$) are slightly more basic than their nucleoside 5'-counterparts ($\text{NP}^{2-/3-/4-}$).⁷ However, it is surprising to find⁸ that no systematic study exists which compares the stabilities of metal ion complexes formed with 2'-deoxyribose nucleotides or ribose nucleotides. Hence, in the present study we are comparing the metal ion-binding properties of the adenine nucleotides shown in Fig.1,⁹ which contain either a ribose (AMP^{2-} , ADP^{3-} , $\text{ATP}^{4-} = \text{NP}^{2-/3-/4-}$) or a 2'-deoxyribose moiety (dAMP^{2-} , dADP^{3-} , $\text{dATP}^{4-} = \text{dNP}^{2-/3-/4-}$).

Figure 1 close to here

As representative metal ions we selected Mg^{2+} , Ni^{2+} and Cu^{2+} ($= \text{M}^{2+}$). Mg^{2+} plays an ubiquitous role in biological systems^{1,10} including ribozymes^{11,12} having a high affinity towards oxygen donor sites like phosphate groups.¹²⁻¹⁴ Ni^{2+} and Cu^{2+} have both a significant affinity towards O and N sites^{13,14} and both play important roles in metabolic as well as toxicologic processes.^{10,15} We have now measured by potentiometric pH titrations the stability constants of the complexes formed between the mentioned three metal ions and the 2'-deoxyadenosine 5'-phosphates ($\text{dNP}^{2-/3-/4-}$), whereas those for the corresponding Zn^{2+} complexes were estimated. The obtained results are then compared with literature data for the adenosine 5'-phosphates ($\text{NP}^{2-/3-/4-}$).¹⁶⁻¹⁸ It turns out that the stabilities of the various $\text{M}(\text{NP})^{0/-/2-}$ and $\text{M}(\text{dNP})^{0/-/2-}$ complexes

are in a first approximation rather similar. However, the stabilities of the $M(dATP)^{2-}$ complexes are somewhat higher and the formation degrees of the macrochelates [see equilibrium (1)] concerning the $M(dATP)^{2-}$ species are slightly more pronounced than it is the case with the $M(ATP)^{2-}$ complexes.



In addition, a comparison of the mentioned $M(AMP)/M(dAMP)$ systems with the previously measured stability constants of some¹⁹ $M(GMP)$ and $M(dGMP)$ complexes^{20,21} (Fig. 1) reveals that macrochelate formation is most pronounced in the $M(dGMP)$ species. This result is in accord with the recent observation⁷ that replacement of the 2'-OH group at the sugar residue by a hydrogen atom makes the N7 site of the guanine residue by about 0.2 pK units more basic. In addition, the (C6)NH₂ group of $AMP^{2-}/dAMP^{2-}$ leads to a steric inhibition for N7 coordination¹⁸ whereas the (C6)O group in $GMP^{2-}/dGMP^{2-}$ does not.

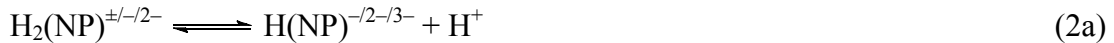
2 Results and discussion

Adenine derivatives undergo self-association due to aromatic-ring stacking of their nucleobases.³ Therefore, all potentiometric pH titrations in this study (25 °C; $I = 0.1$ M, NaNO₃) were carried out at ligand concentrations between 0.13 and 0.6 mM (see Section 4). Under these conditions self-stacking of the adenine nucleotides is negligible¹⁷ and in the present study the properties of the monomeric species were studied, indeed.

2.1 Definition of the equilibrium constants and corresponding results

In the pH range of this study (about 3 to 7.5) the nucleoside 5'-phosphates ($NP^{2-/3-/4-}$) shown in Fig. 1 can accept only two protons. The adenine and the guanine nucleotides can be protonated at their N1 or N7 sites, respectively, and in each case the terminal PO_3^{2-} group can bind a further proton. Hence, the following two deprotonation equilibria (2a) and (3a), the acidity constants of

which were determined recently,⁷ need to be considered:



$$K_{\text{H}_2(\text{NP})}^{\text{H}} = [\text{H}(\text{NP})^{-/2-/3-}][\text{H}^+]/[\text{H}_2(\text{NP})^{\pm/-/2-}] \quad (2b)$$



$$K_{\text{H}(\text{NP})}^{\text{H}} = [\text{NP}^{2-/3-/4-}][\text{H}^+]/[\text{H}(\text{NP})^{-/2-/3-}] \quad (3b)$$

As far as metal ion complex formation is concerned, the experimental data of the potentiometric pH titrations of all M^{2+}/NP systems, where $\text{M}^{2+} = \text{Mg}^{2+}$, Ni^{2+} or Cu^{2+} , are completely described by the acid-base equilibria (2a) and (3a) as well as by the complex-forming equilibria (4a) and (5a), if the evaluation is not carried into the pH range where formation of hydroxo complexes occurs (see also Section 4.2):



$$K_{\text{M}(\text{H};\text{NP})}^{\text{M}} = [\text{M}(\text{H};\text{NP})^{+/0/-}]/([\text{M}^{2+}][\text{H}(\text{NP})^{-/2-/3-}]) \quad (4b)$$



$$K_{\text{M}(\text{NP})}^{\text{M}} = [\text{M}(\text{NP})^{0/-/2-}]/([\text{M}^{2+}][\text{NP}^{2-/3-/4-}]) \quad (5b)$$

Species descriptions such as $\text{M}(\text{H};\text{NP}^{+/0/-})$ [Eqn (4)], where H^+ and NP are separated by a semicolon yet appear within the same parenthesis, indicate that the proton is bound at the ligand without defining its location.

The acidity constant of the connected equilibrium (6a) may be calculated with equation (7):



$$K_{\text{M}(\text{H};\text{NP})}^{\text{H}} = [\text{M}(\text{NP})^{0/-/2-}][\text{H}^+]/[\text{M}(\text{H}(\text{NP})^{+/0/-})] \quad (6b)$$

$$\text{p}K_{\text{M}(\text{H};\text{NP})}^{\text{H}} = \text{p}K_{\text{H}(\text{NP})}^{\text{H}} + \log K_{\text{M}(\text{H};\text{NP})}^{\text{M}} - \log K_{\text{M}(\text{NP})}^{\text{M}} \quad (7)$$

The results obtained now for equilibria (4a), (5a) and (6a), where $\text{NP} = \text{dAMP}^{2-}$, dADP^{3-} or dATP^{4-} , are listed in Table 1 together with the corresponding equilibrium constants for the

M^{2+}/AMP^{2-} , ADP^{3-} and ATP^{4-} systems taken from earlier work,¹⁶⁻¹⁸ as well as some other related data.¹⁸⁻²¹

Table 1 close to here

To the best of our knowledge⁸ the only constant of the dNP systems measured before refers to the $Ni^{2+}/dAMP$ system. Stuehr et al.²² obtained under slightly different conditions (15 °C; $I = 0.1$ M, KNO_3) the following results: $\log K_{Ni(H;dAMP)}^{Ni} = 1.18$ and $\log K_{Ni(dAMP)}^{Ni} = 2.59$. Both values are in excellent agreement with our constants given in Table 1 (entry 2b; columns 7 and 8).

Because for the $M^{2+}/dGMP$ systems, also listed in Table 1, no values for the Ni^{2+} complexes are available²⁰ and because for the 2'-deoxyadenosine 5'-phosphates no stability constants for the Zn^{2+} complexes were determined, we estimated the missing values to obtain for all nucleotides a complete set of data for the four metal ions Mg^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+} . We made the estimations by applying the *Stability Ruler*, proposed by Martin¹⁴ in a quantitative way. For a proof of principle, the stability constant for the $Ni(dGMP)$ complex, which is also known,²¹ is estimated by starting from the Cu^{2+} or the Zn^{2+} complexes and by employing the stability differences (as they follow from the values listed in Table 1) according to the following procedure:

$$\begin{aligned}
 \text{(i)} \quad \log K_{Ni(dGMP)}^{Ni} &= \log K_{Cu(dGMP)}^{Cu} + [\log K_{Ni(GMP)}^{Ni} - \log K_{Cu(GMP)}^{Cu}] \\
 &= (4.05 \pm 0.04) + [(3.50 \pm 0.07) - (3.86 \pm 0.04)] \\
 &= 3.69 \pm 0.09 \\
 \\
 \text{(ii)} \quad \log K_{Ni(dGMP)}^{Ni} &= \log K_{Zn(dGMP)}^{Zn} + [\log K_{Ni(GMP)}^{Ni} - \log K_{Zn(GMP)}^{Zn}] \\
 &= (2.99 \pm 0.05) + [(3.50 \pm 0.07) - (2.83 \pm 0.03)] \\
 &= 3.66 \pm 0.09
 \end{aligned}$$

The results of (i) and (ii) are almost identical and hence, the average of the two calculation procedures is expected to be a reliable estimate: $\log K_{Ni(dGMP)}^{Ni} = 3.68 \pm 0.09$ is listed in Table 1 (entry 1b). Indeed, this value is in excellent accord with the one measured in an independent study where $\log K_{Ni(dGMP)}^{Ni} = 3.60 \pm 0.03$ was determined.²¹

Accordingly, the stability of the $Zn(dATP)^{2-}$ complex can be estimated as follows:

$$\begin{aligned}
 \text{(iii)} \quad \log K_{\text{Zn(dATP)}}^{\text{Zn}} &= \log K_{\text{Cu(dATP)}}^{\text{Cu}} + [\log K_{\text{Zn(ATP)}}^{\text{Zn}} - \log K_{\text{Cu(ATP)}}^{\text{Cu}}] \\
 &= (6.52 \pm 0.05) + [(5.16 \pm 0.06) - (6.34 \pm 0.03)] \\
 &= 5.34 \pm 0.08
 \end{aligned}$$

$$\begin{aligned}
 \text{(iv)} \quad \log K_{\text{Zn(dATP)}}^{\text{Zn}} &= \log K_{\text{Ni(dATP)}}^{\text{Ni}} + [\log K_{\text{Zn(ATP)}}^{\text{Zn}} - \log K_{\text{Ni(ATP)}}^{\text{Ni}}] \\
 &= (5.06 \pm 0.03) + [(5.16 \pm 0.06) - (4.86 \pm 0.05)] \\
 &= 5.36 \pm 0.08
 \end{aligned}$$

The average of (iii) and (iv) gives $\log K_{\text{Zn(dATP)}}^{\text{Zn}} = 5.35 \pm 0.08$, which is also listed in Table 1 (entry 4d).

The above examples illustrate the general applicability of this procedure for the estimation of stability constants if no measured values are available, being the reason why it is presented here in some detail. In an analogous way, the stabilities of the Zn(dAMP) and Zn(dADP)^- complexes were also estimated, the values being also listed in Table 1 (entries 2d and 3d). The estimate for Zn(dAMP) is less satisfying and this is reflected in a considerably larger error limit, though the order of the given stability constant is clearly reasonable.

2.2 Solution structures of the monoprotonated complexes

The stability constants of the monoprotonated complexes formed according to equilibrium (4a) (Table 1, columns 4 and 7) are not very exact with partly rather large error limits because of experimental difficulties (relatively small buffer depression at a low pH). However, despite this shortcoming these complexes definitely exist and hence the question arises, where the proton and where the metal ion is located.

At first one best considers the proton because binding of a metal ion to a protonated ligand commonly leads to an acidification of the ligand-bound proton.²³ Indeed, the acidity constants of the M(H;AMP)^+ and M(H;dAMP)^+ complexes overlap within their error limits (Table 1; columns 6, 9) ($\text{p}K_{\text{M(H;AMP)}}^{\text{H}} \approx 4.6 \pm 0.3$ and $\text{p}K_{\text{M(H;dAMP)}}^{\text{H}} \approx 4.8 \pm 0.3$) and are on average about 1.5 pK units smaller than the acidity constants of H(AMP)^- ($\text{p}K_{\text{H(AMP)}}^{\text{H}} = 6.21$; Table 1, footnote c) and H(dAMP)^- ($\text{p}K_{\text{H(dAMP)}}^{\text{H}} = 6.27$). However, at the same time the acidity constants of the M(H;AMP)^+ and M(H;dAMP)^+ complexes are on average also about 0.8 pK units *larger* than the $\text{p}K_{\text{H}_2(\text{AMP})}^{\text{H}}$ (= 3.84) and $\text{p}K_{\text{H}_2(\text{dAMP})}^{\text{H}}$ (= 3.97) values, which quantify the release of the proton

from the (N1)H⁺ sites (Fig. 1). Because the electron withdrawing effect of a coordinated metal ion always leads to an acidification of a given site, it follows that in the M(H;AMP)⁺ and M(H;dAMP)⁺ complexes the proton must be located at the phosphate group of AMP²⁻ and dAMP²⁻.¹⁷ The analogous reasonings²⁰ for the M(H;GMP)⁺ or M(H;dGMP)⁺ complexes lead to the same result, meaning that also here the protons must be located at the phosphate group of these guanine nucleotides.

Having located the position of the proton, the question now is where the metal ion resides in these monoprotonated nucleotide complexes. A careful evaluation for the M(H;AMP)⁺ complexes has shown¹⁷ that Cu²⁺ as well as the other M²⁺ ions are overwhelmingly coordinated to N7 of the adenine residue whereas the proton resides at the phosphate group. In addition, some of the M²⁺ ions bound at the adenine moiety may form a macrochelate with the P(O)₂(OH)⁻ group. The evaluation of several crystal structures suggests that such an interaction takes place most likely in an outersphere manner.^{9,18,24,25} The species where both, the metal ion and the proton are at the phosphate group is clearly a minority species which occurs only in very small amounts. It is safe to surmise that these earlier conclusions¹⁷ reached for the M(H;AMP)⁺ complexes also hold for the M(H;dAMP)⁺ species.

The corresponding considerations¹⁷ as described above, also hold for the M(H;ADP) and M(H;dADP) complexes (ignoring the Cu²⁺ species, which will be discussed below). Here the average value for the deprotonation of the M(H;ADP) ($pK_{M(H;ADP)}^H \approx 4.65$) and M(H;dADP) ($pK_{M(H;dADP)}^H \approx 4.75$) complexes is about 1.7 pK units below $pK_{H(ADP)}^H$ (= 6.40) or $pK_{H(dADP)}^H$ (= 6.45) and about 0.75 pK units *above* $pK_{H_2(ADP)}^H$ (= 3.92) or $pK_{H_2(dADP)}^H$ (= 4.00). Hence, in all these M(H;ADP) and M(H;dADP) species, the proton must evidently also be located at the diphosphate residue or, more specifically, at the terminal β -phosphate group because this is the most basic site in this residue.

Regarding the location of the metal ion, it was previously concluded¹⁷ that the dominating isomer is the one which has the proton *and* the metal ion at the diphosphate group. However, to some extent also macrochelate formation occurs of the phosphate-bound M²⁺ with N7 of the adenine residue. Only in the case of Cu²⁺ the situation is somewhat more complicated: Roughly 90% of Cu(H;ADP) have H⁺ and Cu²⁺ at the diphosphate group and roughly half of these species

form a macrochelate with N7. The remaining ca 10% have Cu^{2+} at N7 and H^+ at the terminal β -phosphate group. Such a species is not of relevance for all the other metal ion complexes.¹⁷ Overall, if one compares the stability constants listed in Table 1, it is clear that the analogous structures (and approximate percentages) also describe the situation well for the $\text{M}(\text{H};\text{dADP})$ complexes.

In the case of the $\text{M}(\text{H};\text{ATP})^-$ and $\text{M}(\text{H};\text{dATP})^-$ complexes, again ignoring the Cu^{2+} species, the corresponding result is obtained by this analysis,¹⁶ i.e. the proton is located at the terminal γ -phosphate group. Also the metal ion affinity increases with the increasing length of the phosphate chain. With the exception of $\text{Cu}(\text{H};\text{ATP})^-$, it was previously¹⁶ shown for the $\text{M}(\text{H};\text{ATP})^-$ complexes that the metal ions reside at the triphosphate chain with the proton at the terminal γ -phosphate group. Macrochelates are thereby formed to varying extents depending on the kind of metal ion. For $\text{Cu}(\text{H};\text{ATP})^-$ the situation is more complicated.¹⁶ In this case about 50% of the species carry the proton at N1 with Cu^{2+} at the triphosphate residue. The remaining 50% have the proton at the terminal γ -phosphate group with Cu^{2+} also at the triphosphate chain. Cu^{2+} thereby partly interacts also with N7 forming a macrochelate (ca 35%), the remaining part being only phosphate-bound (ca 15%). Overall, the same isomers are expected for the $\text{M}(\text{H};\text{dATP})^-$ complexes in solution; the formation degrees of the macrochelates being possibly somewhat enlarged as is suggested by a comparison of the stability constants of the $\text{M}(\text{H};\text{ATP})^-$ and $\text{M}(\text{H};\text{dATP})^-$ complexes listed under entry 4 in Table 1 (columns 4 and 7).

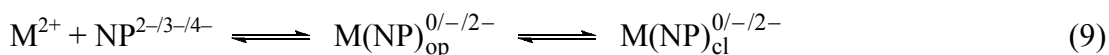
2.3 Proof of macrochelate formation in the $\text{M}(\text{NP})^{0/-2-}$ and $\text{M}(\text{dNP})^{0/-2-}$ complexes

The existence of equilibrium (1) for $\text{M}(\text{AMP})$ complexes²⁶ as well as for $\text{M}(\text{ADP})^-$ (cf. ¹⁷) and $\text{M}(\text{ATP})^{2-}$ (cf. ^{16,27}) species is well established.^{2,3,18,19} As expected, any kind of chelation²⁸ must be reflected in an enhanced complex stability and this also holds for the mentioned cases.^{16,17,26} Of course, macrochelates as indicated in equilibrium (1) will hardly form to 100%. Therefore, we are interested in the formation degree of the macrochelated or 'closed' species, designated as $\text{M}(\text{NP})_{\text{cl}}^{0/-2-}$. The second isomer in equilibrium (1), we refer to as the 'open' species, $\text{M}(\text{NP})_{\text{op}}^{0/-2-}$. This equilibrium is independent of the concentration with the dimensionless equilibrium constant, K_1 , which defines the position of equilibrium (1), and is given by equation

(8):

$$K_I = [M(NP)_{cl}^{0/-/2-}] / [M(NP)_{op}^{0/-/2-}] \quad (8)$$

Taking this into account, equilibrium (5a) may be rewritten as below:



The corresponding stability constant [eqn (5b)] is then defined by equation (10):

$$K_{M(NP)}^M = \frac{[M(NP)_{op}^{0/-/2-}]}{[M^{2+}][NP^{2-/3-/4-}]} \quad (10a)$$

$$= \frac{[M(NP)_{op}^{0/-/2-}] + [M(NP)_{cl}^{0/-/2-}]}{[M^{2+}][NP^{2-/3-/4-}]} \quad (10b)$$

$$= K_{M(NP)op}^M + K_I \cdot K_{M(NP)op}^M \quad (10c)$$

Equation (10c) contains the stability constant of the open isomer shown in equilibrium (1), which is defined in equation (11):

$$K_{M(NP)op}^M = [M(NP)_{op}^{0/-/2-}] / ([M^{2+}][NP^{2-/3-/4-}]) \quad (11)$$

It is evident that any breakdown of the values for $K_{M(NP)}^M$ as indicated by equation (10c) requires values for $K_{M(NP)op}^M$. These cannot directly be measured, in contrast to those for $K_{M(NP)}^M$ [eqn (5a) and (10)]. However, the existence of a linear relationship for families of structurally related ligands between $\log K_{M(L)}^M$ and $pK_{H(L)}^H$ is well known²⁸ and exists also for $\log K_{M(R-MP)}^M$ versus $pK_{H(R-MP)}^H$ (cf. ²⁹) and $\log K_{M(R-DP)}^M$ versus $pK_{H(R-DP)}^H$ plots,³⁰ where R-MP²⁻ represents a simple phosphate monoester or phosphonate ligand²⁹ and R-DP³⁻ a simple diphosphate monoester.³⁰ Hence, R may be any residue which does not affect complex formation. The parameters for the corresponding straight-line equations, which are defined by equation (12),

$$\log K_{M(L)}^M = m \cdot pK_{H(L)}^H + b \quad (12)$$

have been tabulated for L = R-MP²⁻ and R-DP³⁻, i.e., for M(R-MP) (cf. ^{19,29,31}) and M(R-DP)⁻ complexes.³⁰ Hence, with a known pK_a value for the deprotonation of a P(O)₂(OH)⁻ group an expected stability constant can be calculated for any phosphate- or diphosphate-metal ion complex.

Plots of $\log K_{M(R-MP)}^M$ versus $pK_{H(R-MP)}^H$ according to equation (12) are shown in Fig. 2 for the 1:1 complexes of Mg^{2+} , Ni^{2+} and Cu^{2+} with the eight data points (empty circles)^{29,32} defining

Figure 2 close to here

the straight reference lines. The solid points refer to the corresponding $M(AMP)$ and $M(dAMP)$ complexes. Those points representing the Ni^{2+} and Cu^{2+} species are clearly above the reference lines, thus proving an increased stability for these four complexes, whereas the data points for the $Mg(AMP)$ and $Mg(dAMP)$ complexes nearly fit on the line or are only slightly above.

The situation in Fig. 3 for the complexes of diphosphate monoesters ($R-DP^{3-}$) and ADP^{3-} or $dADP^{3-}$ is similar. All six $M(ADP)^{-}$ and $M(dADP)^{-}$ complexes are above the reference lines. Hence, the results displayed in Figures 2 and 3 prove that macrochelates form and that

Figure 3 close to here

equilibrium (1) exists. The different vertical distances of the solid data points to their reference lines thereby illustrate that the extent of macrochelate formation differs for the various systems.

The vertical distances indicated by dotted lines in Figures 2 and 3 evidently correspond to the stability differences $\log \Delta_{M/NP}$ as defined in equation (13):

$$\log \Delta_{M/NP} = \log K_{M(NP)}^M - \log K_{M(NP)op}^M = \log \Delta \quad (13)$$

The stability constants of the $M(NP)^{0/-2-}$ complexes are measured directly and thus known. Instead, the stabilities of the 'open' species [eqn (11)] can be calculated with the previously determined parameters^{29,30} for the straight-line equation (12) and the known acidity constants of the $H(NP)^{-2-}$ species. However, this is true only for the M^{2+} complexes of $GMP^{2-}/dGMP^{2-}$, $AMP^{2-}/dAMP^{2-}$, and $ADP^{3-}/dADP^{3-}$. The corresponding results are summarized according to equation (13) in entries 1 to 3 of Table 2.

Table 2 close to here

For the $M(NTP)^{2-}$ complexes the situation is somewhat different. In the NTP^{4-} species the nucleobase moiety is so distant from the terminal γ -phosphate group that the latter is commonly not influenced by the kind of nucleobase present.^{16,18,33} This means, the pK_a values for most $H(NTP)^{3-}$ species fall into the range of 6.50 ± 0.05 , including $H(CTP)^{3-}$, $H(UTP)^{3-}$ and

H(dTTP)^{3-} . In accord with the fact that no macrochelates are formed, the stability constants of the complexes formed between a given metal ion and these pyrimidine nucleoside 5'-triphosphates are within the error limits identical.^{16,27} Hence, the averages of these values (see Table 2 in ref 27) represent the stabilities of the 'open' isomers in equilibrium (1). These averages can now be used for the evaluation of the M(ATP)^{2-} systems^{16,27} (see Table 2; entry 4, columns 4–6) and other systems like M(ITP)^{2-} or M(GTP)^{2-} .²⁷

Because the acidity constant of H(dATP)^{3-} , $\text{p}K_{\text{H(dATP)}}^{\text{H}} = 6.62 \pm 0.03$, is slightly outside of the mentioned range of 6.50 ± 0.05 , it seems appropriate to take the small acidity difference of $\Delta \text{p}K_{\text{a}} = 0.12$ into account in determining the stabilities of the $\text{M(dATP)}_{\text{op}}^{2-}$ species. Lacking $\log K_{\text{M(R-TP)}}^{\text{M}}$ versus $\text{p}K_{\text{H(R-TP)}}^{\text{H}}$ plots, which cannot be determined because the $\text{p}K_{\text{a}}$ span is too narrow, we decided to apply the slopes m of the $\log K_{\text{M(R-DP)}}^{\text{M}}$ versus $\text{p}K_{\text{H(R-DP)}}^{\text{H}}$ plots.³⁰ This procedure is justified because (i) the slopes for the $\text{M(R-DP)}^-/\text{H(R-DP)}^{2-}$ and $\text{M(R-TP)}^{2-}/\text{H(R-TP)}^{3-}$ systems are expected to be similar and (ii) due to the smallness of $\Delta \text{p}K_{\text{a}} = 0.12$ the resulting correction is also minor. This means, the values listed for $\text{M(dATP)}_{\text{op}}^{2-}$ in column 8 of Table 2 (entry 4) result from adding the small corrections to the values listed for $\text{M(ATP)}_{\text{op}}^{2-}$ in column 5 of Table 2.

A comparison of the $\log \Delta_{\text{M/NP}}$ values listed in Table 2 reveals that the stability enhancement observed due to chelate formation is very similar for the $\text{NP}^{2-/3-/4-}$ and $\text{dNP}^{2-/3-/4-}$ series. In fact, the stability enhancements are within their error limits identical for the complexes of $\text{AMP}^{2-}/\text{dAMP}^{2-}$ and $\text{ADP}^{3-}/\text{dADP}^{3-}$. In the case of the M(NTP)^{2-} complexes the enhancement for the M(dATP)^{2-} species is slightly larger than the one for the M(ATP)^{2-} complexes (note, the error limits given correspond to 3σ). However, a clear difference is observed for the $\text{GMP}^{2-}/\text{dGMP}^{2-}$ systems, as here the stability enhancement of the M(dGMP) complexes is significantly larger than that of the M(GMP) species. This result can be explained by the enhanced basicity of N7 which amounts⁷ to $\Delta \text{p}K_{\text{a}} = 0.21 \pm 0.05$ (for a more detailed discussion see the next section).

2.4 Extent of macrochelate formation in the $\text{M(NP)}^{0/-2-}$ and $\text{M(dNP)}^{0/-2-}$ complexes

From the varying amounts of stability enhancements seen in Figures 2 and 3 and from the differing values listed for $\log \Delta_{\text{M/NP}}$ in Table 2 it follows that the extent of macrochelate

formation in the various complexes also varies. The varying formation degree of the macrochelates indicated in equilibrium (1) can be calculated as follows: The interrelation between the stability enhancement $\log \Delta_{M/NP}$ [eqn (13)] and the dimensionless equilibrium constant K_I [eqn (8)], which defines the position of equilibrium (1), has been established previously,^{28,34,35} and follows from equation (10c):

$$K_I = \frac{K_{M(NP)}^M}{K_{M(NP)op}^M} - 1 = 10^{\log \Delta} - 1 \quad (14)$$

Once K_I is known, the formation degree or percentage of the macrochelated or closed species in equilibrium (1) follows from equation (15):

$$\% M(NP)_{cl}^{0/-/2-} = 100 \cdot K_I / (1 + K_I) \quad (15)$$

Application of the indicated procedure yields the results summarized in Table 3.

Table 3 close to here

The results of Table 3 together with the data collected in Tables 1 and 2 allow many conclusions, several of these are listed below:

(i) If one considers the stabilities of the adenine nucleoside 5'-mono- and 5'-diphosphate complexes, it is evident from Table 1 (columns 5 and 8) that the $M(dNP)^{0/-}$ complexes are only marginally (or not at all) more stable than the $M(NP)^{0/-}$ species. In fact, the slight increase in basicity of the phosphate group parallels the slight stability increase partly observed. In accord herewith, the formation degrees of the macrochelates (Table 3, columns 6 and 9) are within the error limits identical for the $M(AMP)/M(dAMP)$ and $M(ADP)^-/M(dADP)^-$ complex pairs.

(ii) This is different for the $M(ATP)^{2-}/M(dATP)^{2-}$ systems: Here the stability enhancements $\log \Delta_{M/NP}$ are on average about 0.1 log units larger for the $M(dATP)^{2-}$ complexes compared with the $M(ATP)^{2-}$ ones (Table 2, columns 6 and 9). Again, this is also reflected in the formation degrees of the macrochelated species and for all four metal ions it holds $\% M(dATP)_{cl}^{2-} > \% M(ATP)_{cl}^{2-}$ (Table 3, columns 6 and 9).

(iii) Considering an individual case, the formation of macrochelates in the $Mg(NP)^{0/-/2-}$ and $Mg(dNP)^{0/-/2-}$ adenine systems might appear as doubtful. However, taking all results with Mg^{2+} together, it is evident that macrochelates are formed in all instances. However, the

formation degrees with about 15% on average are small, the only exception being Mg(dATP)^{2-} with about 30%.

(iv) Another interesting case, if individual metal ions are considered, is Ni^{2+} : The $\log \Delta_{\text{M/ATP}}$ and $\log \Delta_{\text{M/dATP}}$ values, reflecting the extra affinity towards a nitrogen site, i.e., N7, follow the Irving-Williams series. However, in all other instances the maximum value for $\log \Delta_{\text{M/NP}}$ is always observed with Ni^{2+} , i.e. $\text{Ni(NP)}^{0-}/\text{Ni(dNP)}^{0-}$. This is nicely seen by comparing $\log \Delta_{\text{Ni/AMP}} = 0.61$ and $\log \Delta_{\text{Cu/AMP}} = 0.30$. This deviation from the Irving-Williams series can be explained²⁶ by the different coordination geometries of Ni^{2+} and Cu^{2+} .^{18,26} The Jahn-Teller distorted Cu^{2+} has a strong tendency to coordinate donor atoms (especially N) equatorially. Thus, three positions are left at a phosphate-coordinated Cu^{2+} in an NMP^{2-} complex, but only the *two* cis positions are able to form a macrochelate with N7 for steric reasons. In the octahedral coordination sphere of Ni^{2+} five positions are left after phosphate-coordination and *four* of these are sterically accessible for N7 coordination. Hence, Cu^{2+} backbinding to N7 of the purine ring is statistically disfavored. With increasing distance between the terminal phosphate and N7, the statistical advantage of Ni^{2+} becomes smaller and at the triphosphate level the statistical effect is overruled by other constraints and the order appears as "normal".

(v) It always holds $\text{M(AMP)}_{\text{cl}} < \text{M(GMP)}_{\text{cl}}$. What is the reason for the higher formation degree of macrochelates in the M(GMP) complexes? The intrinsic basicity of N7 can only have a minor effect because 9-methylguanine has a pK_a of 3.11 ± 0.06 (cf. ²³) for its $(\text{N7})\text{H}^+$ site and this value is rather close to the micro acidity constant for the same site in 9-methyladenine, $\text{p}K_{\text{H-N7-N1}}^{\text{N7-N1}} = 2.96 \pm 0.10$.³⁶ Of course, the observed reduced stability depends on the individual metal ion and its affinity towards N sites. Taking the pair Ni(AMP) and Ni(GMP) as an example, this reduction in stability amounts to about 0.9 log unit (Tables 1 and 2), which is to be attributed to the steric inhibition exercised by the $(\text{C6})\text{NH}_2$ group on a metal ion coordinated at N7.³⁷ The $(\text{C6})\text{O}$ group in guanines does not have such an effect. In contrast, it rather promotes complex stability by forming outersphere bonds to a water molecule of the N7-bound metal ion.^{25,38} The conclusion of this observation is that one expects for all complexes of guanine nucleotides higher formation degrees of the macrochelates than for the corresponding adenine nucleotide complexes.

(vi) Finally, but very important, the M(dGMP) complexes are between about 0.1 to 0.2 log unit more stable than the M(GMP) species (Table 1). This remarkable additional stability enhancement, which is also reflected in the $\log \Delta_{M/NMP}$ values as well as in the formation degrees of the macrochelates (Table 3), is to be attributed to the higher basicity of N7 in the 2'-deoxyguanosine residue. Deprotonation of (N7)H⁺ occurs with $\Delta pK_a = 0.21 \pm 0.05$ (Table 1; footnote c) at a higher pH than with the guanosine residue.⁷ Hence, one may expect that in all complexes with 2'-deoxyguanosine 5'-phosphates the extent of macrochelate formation is larger than in the corresponding complexes formed with guanosine 5'-phosphates. Consequently, the information collected in Table 2 can be used for rough estimations of stabilities of complexes where no experimental data is available, e.g. M(dGDP)⁻ and M(dGTP)²⁻ complexes: Such stabilities can be obtained by combining the stabilities of the 'open' species with the stability enhancements observed for the M(dGMP) complexes and by considering the trends reflected in the 2'-deoxyadenosine 5'-phosphate series.³⁹

3 Conclusions

What have we learned from the presented results? If one compares the stabilities of the metal ion complexes formed with the adenosine 5'-phosphates and the 2'-deoxyadenosine 5'-phosphates, it is evident that the overall stabilities of the AMP²⁻/dAMP²⁻ and ADP³⁻/dADP³⁻ pairs differ only very little, the complexes of the deoxy derivatives being slightly (or even not at all) more stable (Table 1). If one compares the extent of macrochelate formation for the same systems, then no differences are observed for a given metal ion (Table 3). In the ATP⁴⁻ or dATP⁴⁻ complexes the situation is only slightly different and in accord with the minor basicity increase observed for dATP⁴⁻.⁷ The complexes of dATP⁴⁻ are somewhat more stable (Table 1) and the formation degree of the macrochelates is a bit enlarged (Table 3).

In contrast, significant differences are observed between the complexing properties of GMP²⁻ and dGMP²⁻: The stability constants of the M(dGMP) complexes are clearly higher than those of the M(GMP) species (Table 1). The same is true for the formation degree of the

macrochelates, which is in accord with the increased basicity of N7 in dGMP²⁻ (Table 3).

Using potentiometric pH titrations, only overall (so-called global) stability constants can be obtained. Hence, different types of macrochelates cannot be distinguished, because the concentration of all complexes, including the sum of all possible macrochelated isomers is measured. However, from studies on M(ATP)²⁻ complexes, including ¹H-NMR shift and spectrophotometric measurements, it is well known that at least two types of macrochelates can form:^{16,40} One in which the phosphate-coordinated metal ion binds innersphere to N7 of the adenine residue and one in which this interaction is of an outersphere type, i.e., with a water molecule between N7 and M²⁺. For example, for Cu(ATP)_{cl}²⁻ it was concluded⁴⁰ that all N7 binding is innersphere, whereas for Mg(ATP)²⁻ only outersphere species form. For Ni(ATP)_{cl}²⁻ it was suggested⁴⁰ that about 30% are N7 innersphere, 25% N7 outersphere, and 45% exist as Ni(ATP)_{op}²⁻ (see also Table 3 for comparison). Similar situations exist for the complexes of guanine nucleotides,^{18,25,27} as well as for NMP²⁻ and NDP³⁻ complexes.^{17,19,25,41} It is thus evident that in this respect considerably more work needs to be done.

The here summarized results, especially those for the M(NMP) complexes, are of relevance for the metal ion-binding properties of nucleic acids: They imply that the N7 sites in single-stranded DNA have a somewhat higher metal ion affinity than the same sites in RNA. Furthermore, the N7 sites of guanine residues are expected to be better metal ion-binding sites than the adenine residues; this applies for both, DNA and RNA.

4 Experimental

4.1 Materials

The disodium salt of 1,2-diaminoethane-N,N,N',N'-tetraacetic acid (Na₂H₂EDTA), and the nitrate salts of Mg²⁺, Ni²⁺, Cu²⁺ (all *pro analysi*) were from Merck KGaA, Darmstadt (Germany). The exact concentrations of the stock solutions of the divalent metal ions were determined by potentiometric pH titrations via their EDTA complexes by measuring the equivalents of protons liberated from H(EDTA)³⁻ upon complex formation. All other materials used in the experiments,

including CO₂-free water and the disodium salts of dAMP, dADP and dATP, were the same as previously.⁷

4.2 Potentiometric pH titrations

The pH titrations were carried out with the reported equipment, which was calibrated as described.⁷ The acidity constants of H₂(dAMP)[±], H₂(dADP)⁻, H₂(dATP)²⁻ were also determined as described⁷ by titrating 50 mL of aqueous 1.67 mM HNO₃ (25 °C; *I* = 0.1 M, NaNO₃) under N₂ with 1 mL of 0.06 M NaOH in the presence and absence of dAP^{2-/3-/4-}, the nucleotide concentration being between 0.13 and 0.6 mM.⁷ The given acidity constants (25 °C; *I* = 0.1 M, NaNO₃) are so-called practical, mixed, or Brønsted constants,^{34,42} which may be converted into the corresponding concentration constants by subtracting 0.02 from the listed p*K*_a values.⁴² The stability constants of the complexes presented are, as usual, concentration constants. All measurements were performed under the same conditions as used for the acidity constants,⁷ but NaNO₃ was partly (Mg²⁺, Ni²⁺ and Cu²⁺) or, in some experiments with Mg²⁺, fully replaced by M(NO₃)₂.

The metal-to-ligand ratios in the various titrations with dAMP were 63:1, 51:1 and 46:1 for Mg²⁺; 32:1, 27:1 and 22:1 for Ni²⁺; and 6:3, 3.5:1 and 1.5:1 for Cu²⁺. The corresponding ratios for dADP were 7:1, 2.9:1 and 1.3:1 for Mg²⁺; and 2.0:1, 1.9:1 and 1.3:1 for Ni²⁺ and Cu²⁺. In the case of dATP the metal-to-ligand ratios were 7:1, 2.0:1, 1.5:1 and 1.2:1 for Mg²⁺, as well as 1.7:1, 1.5:1, 1.2:1, 1.05:1 for Ni²⁺ and Cu²⁺. It should be mentioned that the calculated stability constants for the M²⁺ complexes showed no dependence on the excess of M²⁺ used. In addition the fitting procedures of the experimental data gave no indication of the formation of M₂(dAP)^{2+/-0} or any other M²⁺/ligand species.

The data were collected every 0.1 pH unit starting from a formation degree of the M(H;dAP)^{+0/-} species of about 3 to 30%, depending on the metal ion and ligand under consideration. The upper limit was given by either the onset of the hydrolysis of M(aq)²⁺, which was evident from the titrations without ligand, or by a formation degree of about 90% for the M(dAP)^{0/-2-} species. Representative examples for the pH ranges employed with dAMP are 3.1–7.2 for Mg²⁺, 3.3–6.8 for Ni²⁺ and 3.3–5.6 for Cu²⁺. For the dADP and dATP systems the

corresponding pH ranges are 3.6–7.7 and 3.6–8.0 for Mg^{2+} , 3.6–7.7 and 3.3–6.9 for Ni^{2+} , and 3.0–5.8 and 3.1–5.4 for Cu^{2+} , respectively.

The stability constants of the Mg^{2+} , Ni^{2+} and Cu^{2+} systems were calculated as described previously⁴³ by taking into account the species H^+ , $\text{H}_2(\text{dAP})^{\pm/-/2-}$, $\text{H}(\text{dAP})^{-2-/3-}$, $\text{dAP}^{2-/3-/4-}$, M^{2+} , $\text{M}(\text{H;dAP})^{+/0/-}$ and $\text{M}(\text{dAP})^{0/-/2-}$. The final results for the stability constants of the complexes are the averages of three independent titrations for the dAMP and dADP systems, whereas for dATP four independent titrations with each metal ion have been performed.

Since it is wellknown that divalent metal ions promote the dephosphorylation of NTPs,⁴⁴ this must also be surmised for dNTPs as well as dNDPs. Therefore, the nucleotide solutions were mixed with the metal ion solutions just before the titration started to minimize in this way any dephosphorylation of the nucleotides. It needs to be added that no additional H^+ were liberated showing that during the time of the titrations no nucleotide hydrolysis occurred.

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Table 1 Comparison of the logarithms of the stability constants [eqn (4) and (5)] as determined by potentiometric pH titrations of several metal ion complexes formed with nucleoside 5'-phosphates containing a ribose (NP^{2-/3-/4-}) or a 2'-deoxyribose moiety (dNP^{2-/3-/4-}), together with the negative logarithms for the acidity constants of the monoprotonated M(H;NP/dNP)^{+0/-} complexes [eqn (6) and (7)] (25 °C; *I* = 0.1 M, NaNO₃)^{a,b,c}

No.	Nucleotide NP/dNP	M ²⁺	NP ^{2-/3-/4-}			dNP ^{2-/3-/4-}		
			log $K_{M(H;NP)}^M$	log $K_{M(NP)}^M$	pK _{M(H;NP)} ^H	log $K_{M(H;dNP)}^M$	log $K_{M(dNP)}^M$	pK _{M(H;dNP)} ^H
1a	GMP ²⁻ /dGMP ²⁻	Mg ²⁺	0.3 ± 0.3	1.73 ± 0.03	4.8 ± 0.3	0.5 ± 0.3	1.81 ± 0.04	5.0 ± 0.3
1b		Ni ²⁺	2.0 ± 0.3	3.50 ± 0.07	4.75 ± 0.3		3.68 ± 0.09 ^d	
1c		Cu ²⁺	2.5 ± 0.3	3.86 ± 0.04	4.9 ± 0.3	2.81 ± 0.06	4.05 ± 0.04	5.05 ± 0.07
1d		Zn ²⁺	1.4 ± 0.2	2.83 ± 0.03	4.8 ± 0.2	1.76 ± 0.06	2.99 ± 0.05	5.06 ± 0.08
2a	AMP ²⁻ /dAMP ²⁻	Mg ²⁺	0.0 ± 0.3	1.62 ± 0.04	4.6 ± 0.3	0.1 ± 0.2	1.68 ± 0.03	4.7 ± 0.2
2b		Ni ²⁺	1.05 ± 0.15	2.55 ± 0.04	4.71 ± 0.16	1.20 ± 0.15	2.50 ± 0.08	4.97 ± 0.17
2c		Cu ²⁺	1.5 ± 0.2	3.17 ± 0.02	4.54 ± 0.20	1.8 ± 0.2	3.34 ± 0.10	4.73 ± 0.22
2d		Zn ²⁺	0.8 ± 0.3	2.38 ± 0.07	4.63 ± 0.31		2.44 ± 0.16 ^d	
3a	ADP ³⁻ /dADP ³⁻	Mg ²⁺	1.68 ± 0.10	3.36 ± 0.03	4.72 ± 0.10	1.70 ± 0.15	3.38 ± 0.07	4.77 ± 0.17
3b		Ni ²⁺	2.26 ± 0.15	3.93 ± 0.02	4.73 ± 0.15	2.28 ± 0.20	3.98 ± 0.05	4.75 ± 0.21
3c		Cu ²⁺	2.77 ± 0.16	5.61 ± 0.03	3.56 ± 0.16	2.8 ± 0.2	5.61 ± 0.07	3.64 ± 0.21
3d		Zn ²⁺	2.31 ± 0.20	4.28 ± 0.05	4.43 ± 0.21		4.31 ± 0.09 ^d	
4a	ATP ⁴⁻ /dATP ⁴⁻	Mg ²⁺	2.42 ± 0.08	4.29 ± 0.03	4.60 ± 0.08	2.49 ± 0.13	4.41 ± 0.05	4.70 ± 0.14
4b		Ni ²⁺	2.86 ± 0.11	4.86 ± 0.05	4.47 ± 0.12	2.96 ± 0.10	5.06 ± 0.03	4.52 ± 0.11
4c		Cu ²⁺	3.59 ± 0.08	6.34 ± 0.03	3.73 ± 0.09	3.82 ± 0.09	6.52 ± 0.05	3.92 ± 0.11
4d		Zn ²⁺	2.86 ± 0.11	5.16 ± 0.06	4.17 ± 0.13		5.36 ± 0.08 ^d	

^a The error limits are *three times* the standard error of the mean value (3σ) or the sum of the probable systematic errors, whichever is larger. The error limits (3σ) of the derived data, in the present case for columns 6 and 9 (and in the text as well), were calculated according to the error propagation after Gauss. ^b The listed constants (see also Section 4.2) are taken for GMP from ref. 19, for dGMP from ref. 20, for AMP and ADP from ref. 17, and for ATP from ref. 16 (see also refs 2,18). All the values given for dAMP, dADP and dATP are from this study. ^c Acidity constants as defined by eqn (2) and (3) for the twofold protonated nucleotides given above; the values are taken from the list assembled in ref. 7: pK_{H₂(GMP)}^H = 2.48 ± 0.04, pK_{H(GMP)}^H = 6.25 ± 0.02; pK_{H₂(dGMP)}^H = 2.69 ± 0.03, pK_{H(dGMP)}^H = 6.29 ± 0.01; pK_{H₂(AMP)}^H = 3.84 ± 0.02, pK_{H(AMP)}^H = 6.21 ± 0.01; pK_{H₂(dAMP)}^H = 3.97 ± 0.02, pK_{H(dAMP)}^H = 6.27 ± 0.04; pK_{H₂(ADP)}^H = 3.92 ± 0.02, pK_{H(ADP)}^H = 6.40 ± 0.01; pK_{H₂(dADP)}^H = 4.00 ± 0.03, pK_{H(dADP)}^H = 6.45 ± 0.02; pK_{H₂(ATP)}^H = 4.00 ± 0.01, pK_{H(ATP)}^H = 6.47 ± 0.01; pK_{H₂(dATP)}^H = 4.14 ± 0.02 and pK_{H(dATP)}^H = 6.62 ± 0.03 (25 °C; *I* = 0.1 M, NaNO₃). ^d Estimated value; see text in Section 2.1.

Table 2 Comparison of the overall stability constants of the $M(NP)^{0/-/2-}$ and $M(dNP)^{0/-/2-}$ complexes [eqn (10)] with those of the corresponding 'open' species, $M(NP)_{op}^{0/-/2-}$ and $M(dNP)_{op}^{0/-/2-}$ [eqn (11)], having only a M^{2+} -phosphate coordination, together with the resulting stability differences $\log \Delta_{M/NP}$ [eqn (13)] (25 °C; $I = 0.1$ M, $NaNO_3$)^a

No.	Nucleotide NP/dNP	M^{2+}	$NP^{2-/3-/4-}$			$dNP^{2-/3-/4-}$		
			$\log K_{M(NP)}^M$	$\log K_{M(NP)op}^M$	$\log \Delta_{M/NP}$	$\log K_{M(dNP)}^M$	$\log K_{M(dNP)op}^M$	$\log \Delta_{M/dNP}$
1a	GMP ²⁻ /dGMP ²⁻	Mg ²⁺	1.73 ± 0.03	1.57 ± 0.03	0.16 ± 0.04	1.81 ± 0.04	1.58 ± 0.03	0.23 ± 0.05
1b		Ni ²⁺	3.50 ± 0.07	1.95 ± 0.05	1.55 ± 0.09	3.68 ± 0.09	1.96 ± 0.05	1.72 ± 0.10
1c		Cu ²⁺	3.86 ± 0.04	2.89 ± 0.06	0.97 ± 0.07	4.05 ± 0.04	2.91 ± 0.06	1.14 ± 0.07
1d		Zn ²⁺	2.83 ± 0.03	2.14 ± 0.06	0.69 ± 0.07	2.99 ± 0.05	2.15 ± 0.06	0.84 ± 0.08
2a	AMP ²⁻ /dAMP ²⁻	Mg ²⁺	1.62 ± 0.04	1.56 ± 0.03	0.06 ± 0.05	1.68 ± 0.03	1.58 ± 0.03	0.10 ± 0.04
2b		Ni ²⁺	2.55 ± 0.04	1.94 ± 0.05	0.61 ± 0.06	2.50 ± 0.08	1.96 ± 0.05	0.54 ± 0.09
2c		Cu ²⁺	3.17 ± 0.02	2.87 ± 0.06	0.30 ± 0.06	3.34 ± 0.10	2.90 ± 0.06	0.44 ± 0.12
2d		Zn ²⁺	2.38 ± 0.07	2.13 ± 0.06	0.25 ± 0.09	2.44 ± 0.16	2.15 ± 0.06	0.29 ± 0.17
3a	ADP ³⁻ /dADP ³⁻	Mg ²⁺	3.36 ± 0.03	3.30 ± 0.03	0.06 ± 0.04	3.38 ± 0.07	3.32 ± 0.03	0.06 ± 0.08
3b		Ni ²⁺	3.93 ± 0.02	3.54 ± 0.06	0.39 ± 0.06	3.98 ± 0.05	3.57 ± 0.06	0.41 ± 0.08
3c		Cu ²⁺	5.61 ± 0.03	5.27 ± 0.04	0.34 ± 0.05	5.61 ± 0.07	5.33 ± 0.04	0.28 ± 0.08
3d		Zn ²⁺	4.28 ± 0.05	4.12 ± 0.03	0.16 ± 0.06	4.31 ± 0.09	4.17 ± 0.03	0.14 ± 0.09
4a	ATP ⁴⁻ /dATP ⁴⁻	Mg ²⁺	4.29 ± 0.03	4.21 ± 0.04	0.08 ± 0.05	4.41 ± 0.05	4.27 ± 0.04 ^b	0.14 ± 0.06
4b		Ni ²⁺	4.86 ± 0.05	4.50 ± 0.03	0.36 ± 0.06	5.06 ± 0.03	4.59 ± 0.03 ^b	0.47 ± 0.04
4c		Cu ²⁺	6.34 ± 0.03	5.86 ± 0.03	0.48 ± 0.04	6.52 ± 0.05	5.96 ± 0.03 ^{b,c}	0.56 ± 0.06
4d		Zn ²⁺	5.16 ± 0.06	5.02 ± 0.02	0.14 ± 0.06	5.36 ± 0.08	5.15 ± 0.02 ^b	0.21 ± 0.08

^a For the error limits and the source of the data see footnotes *a* and *b* of Table 1, respectively (see also Section 2.1). The values in columns 4 and 7 are from Table 1 (columns 5 and 8). ^b See the second to the last paragraph in Section 2.3 where it is described how these values were obtained.

^c Because of the square-pyramidally distorted coordination sphere of Cu²⁺ and the presence of two phosphate groups in dADP³⁻ instead of three in dATP⁴⁻, a statistical factor of 2/3 was applied to the slope (see text) and $m = 0.855$ was used in the calculation.

Table 3 Extent of macrochelate formation in $M(NP)^{0/-/2-}$ and $M(dNP)^{0/-/2-}$ complexes [eqn (1)] as calculated from the stability enhancements $\log \Delta_{M/NP}$ and $\log \Delta_{M/dNP}$ [eqn (13)] and quantified by the dimensionless equilibrium constant K_I [eqn (8,14)] and the percentage of the macrochelated isomers $M(NP)_{cl}^{0/-/2-}$ and $M(dNP)_{cl}^{0/-/2-}$ [eqn (15)] in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$)^a

No.	Nucleotide NP/dNP	M^{2+}	$NP^{2-/3-/4-}$			$dNP^{2-/3-/4-}$		
			$\log \Delta_{M/NP}$	$K_{I/NP}$	% $M(NP)_{cl}$	$\log \Delta_{M/dNP}$	$K_{I/dNP}$	% $M(dNP)_{cl}$
1a	GMP ²⁻ /dGMP ²⁻	Mg ²⁺	0.16 ± 0.04	0.45 ± 0.13	31 ± 6	0.23 ± 0.05	0.70 ± 0.20	41 ± 7
1b		Ni ²⁺	1.55 ± 0.09	34.48 ± 7.35	97 ± 1	1.72 ± 0.10	51.48 ± 12.08	98 ± 1
1c		Cu ²⁺	0.97 ± 0.07	8.33 ± 1.50	89 ± 2	1.14 ± 0.07	12.80 ± 2.22	93 ± 1
1d		Zn ²⁺	0.69 ± 0.07	3.90 ± 0.79	80 ± 3	0.84 ± 0.08	5.92 ± 1.27	86 ± 3
2a	AMP ²⁻ /dAMP ²⁻	Mg ²⁺	0.06 ± 0.05	0.15 ± 0.13	13 ± 10	0.10 ± 0.04	0.26 ± 0.12	21 ± 7
2b		Ni ²⁺	0.61 ± 0.06	3.07 ± 0.56	75 ± 3	0.54 ± 0.09	2.47 ± 0.72	71 ± 6
2c		Cu ²⁺	0.30 ± 0.06	1.00 ± 0.28	50 ± 7	0.44 ± 0.12	1.75 ± 0.76	64 ± 10
2d		Zn ²⁺	0.25 ± 0.09	0.78 ± 0.37	44 ± 12	0.29 ± 0.17	0.95 ± 0.76	49 ± 20
3a	ADP ³⁻ /dADP ³⁻	Mg ²⁺	0.06 ± 0.04	0.15 ± 0.11	13 ± 8	0.06 ± 0.08	0.15 ± 0.21	13 ± 16
3b		Ni ²⁺	0.39 ± 0.06	1.45 ± 0.34	59 ± 6	0.41 ± 0.08	1.57 ± 0.47	61 ± 7
3c		Cu ²⁺	0.34 ± 0.05	1.19 ± 0.25	54 ± 5	0.28 ± 0.08	0.91 ± 0.35	48 ± 10
3d		Zn ²⁺	0.16 ± 0.06	0.45 ± 0.20	31 ± 10	0.14 ± 0.09	0.38 ± 0.29	28 ± 15
4a	ATP ⁴⁻ /dATP ⁴⁻	Mg ²⁺	0.08 ± 0.05	0.20 ± 0.14	17 ± 10	0.14 ± 0.06	0.38 ± 0.19	28 ± 10
4b		Ni ²⁺	0.36 ± 0.06	1.29 ± 0.32	56 ± 6	0.47 ± 0.04	1.95 ± 0.27	66 ± 3
4c		Cu ²⁺	0.48 ± 0.04	2.02 ± 0.28	67 ± 3	0.56 ± 0.06	2.63 ± 0.50	69 ± 4
4d		Zn ²⁺	0.14 ± 0.06	0.38 ± 0.19	28 ± 10	0.21 ± 0.08	0.62 ± 0.30	38 ± 11

^a For the error limits and the calculation procedure see footnote *a* of Table 1 and refs 28,34, respectively. The values in columns 4 and 7 are from Table 2 (columns 6 and 8).

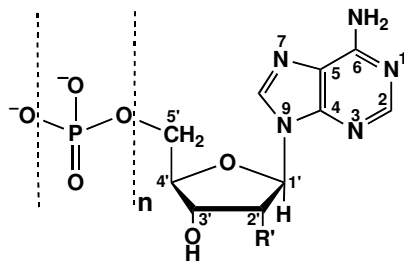
Legends for the Figures

Fig. 1 Chemical structures of (2'-deoxy)adenosine 5'-monophosphate (AMP^{2-} ; dAMP^{2-}), (2'-deoxy)adenosine 5'-diphosphate (ADP^{3-} ; dADP^{3-}) and (2'-deoxy)adenosine 5'-triphosphate (ATP^{4-} ; dATP^{4-}) (top part), and of (2'-deoxy)guanosine 5'-monophosphate (GMP^{2-} ; dGMP^{2-}) (lower part). All nucleotides are shown in their dominating *anti* conformation.^{7,9} The phosphate groups of the nucleoside 5'-triphosphates ($\text{NTP}^{4-} = \text{ATP}^{4-}$, dATP^{4-}) are labeled α , β and γ , whereby γ refers to the terminal phosphate group (not shown). Analogously, the phosphate groups of the NDPs are labeled as α and β , β being the terminal one. The abbreviation $\text{NP}^{2-/3-/4-}$ refers to all nucleotides mentioned above. If the 2'-deoxy compounds need to be distinguished, in addition the abbreviation $\text{dNP}^{2-/3-/4-}$ is used.

Fig. 2 Evidence for an enhanced stability of some $\text{M}(\text{AMP})$ and $\text{M}(\text{dAMP})$ complexes (●), based on the relationship between $\log K_{\text{M}(\text{R-MP})}^{\text{M}}$ and $\text{p}K_{\text{H}(\text{R-MP})}^{\text{H}}$ for $\text{M}(\text{R-MP})$ complexes of some simple phosphate monoester and phosphonate ligands (R-MP^{2-}) (○): 4-nitrophenyl phosphate (NPhP^{2-}), phenyl phosphate (PhP^{2-}), uridine 5'-monophosphate (UMP^{2-}), D-ribose 5-monophosphate (RibMP^{2-}), thymidine [= 1-(2'-deoxy- β -D-ribofuranosyl)thymine] 5'-monophosphate (dTMP^{2-}), *n*-butyl phosphate (BuP^{2-}), methanephosphonate (MeP^{2-}), and ethanephosphonate (EtP^{2-}) (from left to right). The least-squares lines²⁹ [eqn (12)] are drawn through the corresponding eight data sets (○) taken from ref. 32 for the phosphate monoesters and from ref. 29 for the phosphonates. The data points due to the M^{2+}/AMP and $\text{M}^{2+}/\text{dAMP}$ systems (●) are based on the values listed in Table 1. The vertical broken lines emphasize the stability differences from the reference lines; they equal $\log \Delta_{\text{M/NP}}$ as defined in equation (13) (see also Table 2, columns 6 and 9). All the plotted equilibrium constants refer to aqueous solutions at 25 °C and $I = 0.1 \text{ M}$ (NaNO_3).

Fig. 3 Evidence for an enhanced stability of the Mg^{2+} , Ni^{2+} and Cu^{2+} complexes formed with ADP^{3-} (●) and dADP^{3-} (◆), based on the relationship between $\log K_{\text{M}(\text{R-DP})}^{\text{M}}$ and $\text{p}K_{\text{H}(\text{R-DP})}^{\text{H}}$ for the simple $\text{M}(\text{R-DP})^-$ complexes (○), where $\text{R-DP}^{3-} = \text{phenyl diphosphate} (\text{PhDP}^{3-})$, methyl

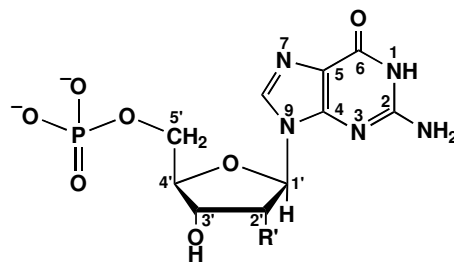
diphosphate (MeDP^{3-}), uridine 5'-diphosphate (UDP^{3-}), cytidine 5'-diphosphate (CDP^{3-}), thymidine [= 1-(2'-deoxy- β -D-ribofuranosyl)thymine] 5'-diphosphate (dTDP^{3-}) and *n*-butyl diphosphate (BuDP^{3-}) (from left to right). The least-squares lines [eqn (12)] are drawn through the indicated six (in the case of Cu^{2+} five) data sets; the corresponding equilibrium constants are from ref. 30. The data points due to the M^{2+}/ADP and $\text{M}^{2+}/\text{dADP}^{3-}$ systems (●) are based on the values listed in Table 1. The vertical broken lines emphasize the stability differences from the reference lines; they equal $\log \Delta_{\text{M/NP}}$ as defined in equation (13) (see also Table 2, columns 6 and 9). All the plotted equilibrium constants refer to aqueous solutions at 25 °C and $I = 0.1 \text{ M}$ (NaNO_3).



AMP²⁻: $n = 1$; $R' = OH$
dAMP²⁻: $n = 1$; $R' = H$

ADP³⁻: $n = 2$; $R' = OH$
dADP³⁻: $n = 2$; $R' = H$

ATP⁴⁻: $n = 3$; $R' = OH$
dATP⁴⁻: $n = 3$; $R' = H$



GMP²⁻: $R' = OH$
dGMP²⁻: $R' = H$

Figure 1

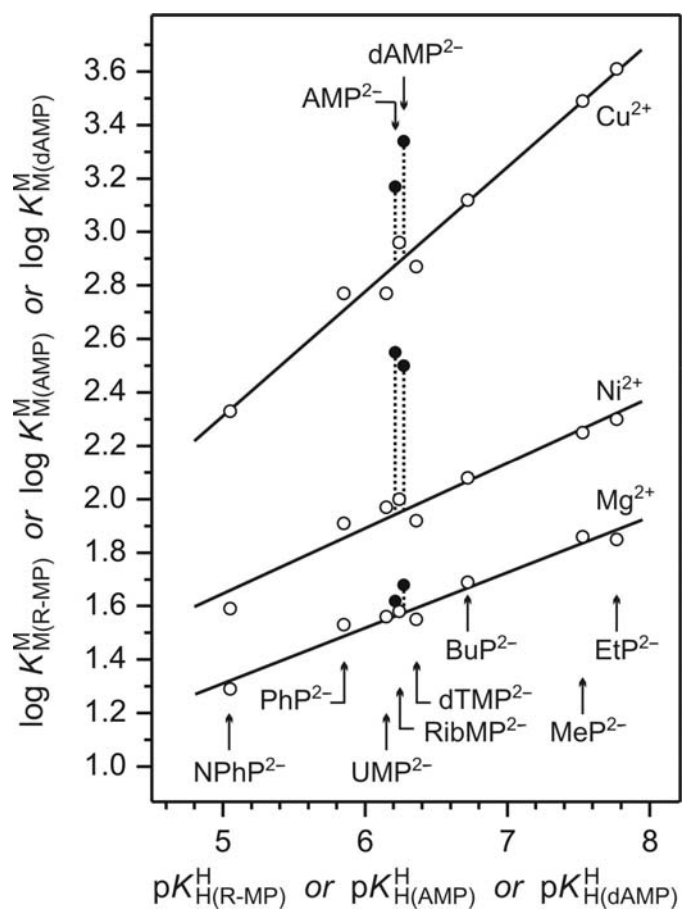


Figure 2

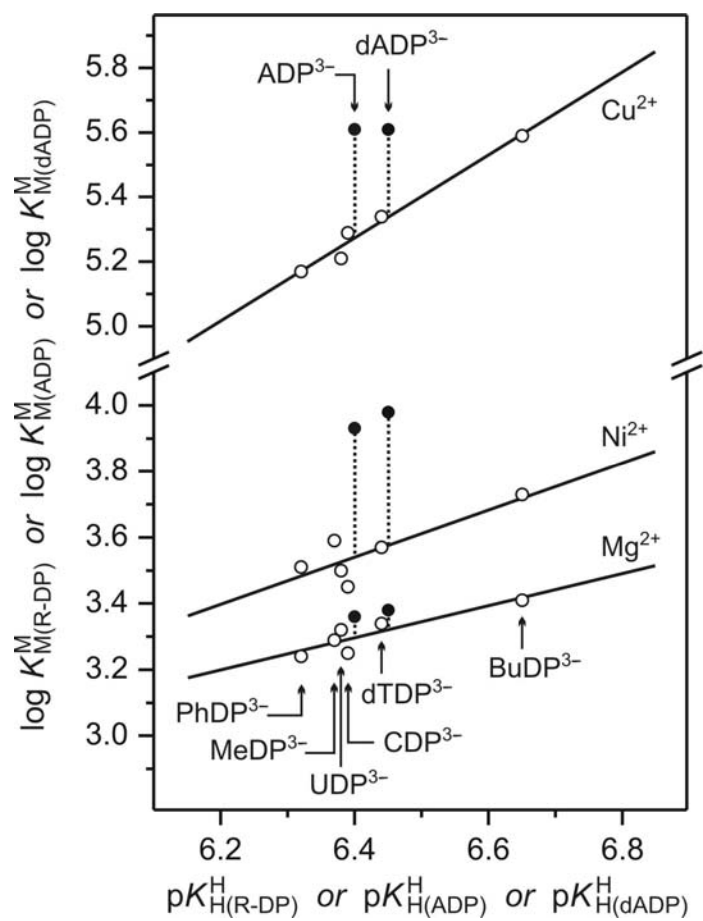


Figure 3